CLAIMS

1. A method for quantifying one or more peptides in a peptide mixture, comprising:

receiving a first peptide mixture containing a plurality of peptides;

separating one or more of the plurality of peptides of the first peptide mixture over a period of time;

mass-to-charge analyzing one or more of the separated peptides of the first peptide mixture at a particular time in the period of time;

calculating an abundance of one or more of the mass analyzed peptides of the first peptide mixture; and

calculating a relative quantity for the one or more mass analyzed peptides of the first peptide mixture by comparing the calculated abundance of the one or more mass analyzed peptides of the first peptide mixture with an abundance of one or more peptides in a reference sample, the reference sample being external to the first peptide mixture.

2. The method of claim 1, wherein:

receiving a first peptide mixture containing a plurality of peptides comprises digesting a first polypeptide sample to generate the first peptide mixture.

3. The method of claim 2, further comprising:

preparing the reference sample by digesting a second polypeptide sample;

separating one or more peptides from the digested second polypeptide sample;

mass analyzing the separated peptides from the digested second polypeptide sample; and

calculating an abundance of one or more of the mass analyzed peptides from the second polypeptide sample;

wherein calculating a relative quantity for the one or more mass analyzed peptides of the first peptide mixture comprises comparing the calculated abundance of the one or more mass analyzed peptides of the first peptide mixture with the calculated abundance of one or more corresponding mass analyzed peptides from the second polypeptide sample.

4. The method of claim 1, wherein:

separating one or more peptides comprises separating the one or more peptides by liquid chromatography.

5. The method of claim 4, wherein:

separating one or more peptides comprises isolating a liquid chromatography eluent at the particular time; and

mass analyzing one or more of the separated peptides of the first peptide mixture comprises mass analyzing one or more peptides in the isolated eluent.

6. The method of claim 1, further comprising:

identifying one or more peptides of the first peptide mixture.

7. The method of claim 6, wherein:

identifying one or more peptides of the first peptide mixture comprises identifying one or more of the separated peptides based on mass analysis information.

8. The method of claim 7, wherein:

mass analyzing one or more of the separated peptides comprises fragmenting an ion derived from a peptide of the one or more separated peptides and mass analyzing fragments of the ion; and

identifying one or more peptides in the first sample comprises searching a sequence database based on mass analysis information for the fragments.

9. The method of claim 4, wherein:

calculating an abundance of one or more of the mass analyzed peptides comprises reconstructing a chromatogram peak for a peptide based on mass analysis information for the peptide.

10. The method of claim 9, wherein:

calculating an abundance for a peptide comprises calculating an abundance for a peptide based on a reconstructed chromatogram peak area for the peptide.

11. The method of claim 10, wherein:

calculating the abundance for a peptide comprises calculating an abundance for a peptide using only chromatogram peaks located within a threshold distance in the reconstructed chromatogram of the particular time.

12. The method of claim 10, wherein:

calculating a relative quantity for the one or more mass analyzed peptides comprises comparing an abundance calculated by reconstructing a chromatogram peak area for a peptide of the first peptide mixture with an abundance calculated by reconstructing a chromatogram peak area for a peptide in the reference sample.

13. The method of claim 2, further comprising:

normalizing the calculated abundance of the one or more mass analyzed peptides of the first peptide mixture.

14. The method of claim 13, wherein:

normalizing the calculated abundance comprises normalizing the calculated abundance based on an internal standard including one or more peptides added to the first polypeptide sample.

15. The method of claim 13, wherein:

normalizing the calculated abundance comprises normalizing the calculated abundance based on an external standard including one or more peptides.

16. The method of claim 2, further comprising:

identifying a plurality of peptides of the first peptide mixture based on the mass analyzing;

wherein calculating a relative quantity for the one or more mass analyzed peptides comprises calculating a relative quantity for each of the identified peptides.

17. The method of claim 16, further comprising:

normalizing calculated abundances for each of the identified peptides by calculating a correction factor based on reconstructed chromatogram peak areas for a set of peptides in the first peptide mixture, each peptide in the set of peptides having constant

chromatogram peak areas over a plurality of experiments, and applying the correction factor to the calculated abundance for each of the identified peptides.

18. The method of claim 1, wherein:

the mass analyzing and calculating steps are performed to identify and calculate relative quantities for every peptide in the first peptide mixture in a single automated experiment.

19. The method of claim 1, wherein:

the one or more of the separated peptides that are subjected to the mass-to-charge analyzing and calculating steps are naturally occurring peptides.

20. The method of claim 19, wherein:

the one or more peptides in the reference sample are naturally occurring peptides.

21. The method of claim 1, wherein:

mass-to-charge analyzing one or more of the separated peptides and calculating an abundance of one or more of the mass analyzed peptides comprises mass-to-charge analyzing and calculating an abundance for one or more arbitrary peptides of the first peptide mixture.

22. The method of claim 1, wherein:

the separating, mass-to-charge analyzing, and calculating steps are not constrained to a particular amino acid composition of the subject peptides.

23. A method of quantifying one or more peptides in a mixture, comprising:

digesting a protein sample to generate a mixture of peptides;

separating one or more peptides of the mixture of peptides using liquid chromatography;

mass analyzing one or more of the separated peptides;

identifying one or more of the mass analyzed peptides based on mass spectra for the peptides;

calculating chromatogram peak areas for the identified peptides;

calculating chromatogram peak areas for one or more proteins corresponding to the identified peptides based on the calculated peak areas for the corresponding peptides;

normalizing the chromatogram peak area for the protein based on a chromatogram peak area for an internal standard; and

determining a relative quantity for a protein of the one or more of the proteins by comparing the normalized chromatogram peak area for the protein to a chromatogram peak area for a corresponding protein in a reference sample.

24. An apparatus for quantifying one or more peptides in a peptide mixture, comprising:

means for receiving a first peptide mixture containing a plurality of peptides;

means for separating one or more of the plurality of peptides of the first peptide mixture over a period of time;

means for mass analyzing one or more of the separated peptides of the first peptide mixture at a particular time in the period of time;

means for calculating an abundance of one or more of the mass analyzed peptides of the first peptide mixture;

means for calculating a relative quantity for the one or more mass analyzed peptides of the first peptide mixture by comparing the calculated abundance of the one or more mass analyzed peptides of the first peptide mixture with an abundance of one or more peptides in a reference sample which is external to first peptide mixture.

25. The apparatus of claim 24, wherein:

the means for calculating the abundance and the means for calculating the relative quantity are the same.

26. The apparatus of claim 24, wherein

the means for mass analyzing is an ion trap mass spectrometer, a triple quadrupole mass spectrometer, a quadrupole time-of-flight mass spectrometer, a trap time-of-flight mass spectrometer, a Fourier transform ion cyclotron resonance mass spectrometer, a post-source decay time-of-flight mass spectrometer, time-of-flight - time-of-flight mass spectrometer

or an orbitrap mass spectrometer.

27. The apparatus of claim 24, wherein:

the means for separating comprises at least one of liquid chromatography, gas chromatography, electropheresis and capillary electrophoresis.

28. The apparatus of claim 27, wherein:

the means for separating comprises at least two dimensions of separation.

29. The apparatus of claim 24, wherein:

the means for calculating comprises a computer system.

30. The apparatus of claim 24, further comprising:

means for receiving at least one additional peptide mixture.

31. The apparatus of claim 30, wherein:

the at least one additional peptide mixture comprises a reference sample.

32. The apparatus of claim 24, wherein:

the means for calculating an abundance further comprises reference information.

33. The apparatus of claim 24, wherein:

the means for mass-to-charge analyzing and the means for calculating are configured to mass-to-charge analyze and calculate an abundance for are naturally occurring peptides.

34. The apparatus of claim 33, wherein:

the means for calculating is configured to compare the calculated abundance of the one or more mass analyzed peptides with an abundance of one or more naturally occurring peptides in a reference sample.

34. The apparatus of claim 24, wherein:

the means for mass-to-charge analyzing and the means for calculating are configured to mass-to-charge analyze and calculate an abundance for one or more arbitrary peptides of the first peptide mixture.

35. The apparatus of claim 24, wherein:

the means for separating, mass-to-charge analyzing, and calculating steps are configured to separate, mass-to-charge analyze and calculate an abundance for one or more peptides independent of a particular amino acid composition of the subject peptides.

36. A computer program product on a computer-readable medium for quantifying one or more peptides in a first peptide mixture, the product comprising instructions operable to cause a programmable processor to:

receive separation information representing a separation of one or more of a plurality of peptides of a first peptide mixture over a period of time;

receive mass-to-charge analysis information for one or more of the separated peptides of the first peptide mixture at a particular time in the period of time;

calculate an abundance of one or more of the mass analyzed peptides of the first peptide mixture; and

calculate a relative quantity for the one or more mass analyzed peptides of the first peptide mixture by comparing the calculated abundance of the one or more mass analyzed peptides of the first peptide mixture with an abundance of one or more peptides in a reference sample, the reference sample being external to the first peptide mixture.

37. A computer program product on a computer-readable medium for quantifying one or more peptides in a first peptide mixture, the product comprising instructions operable to cause a programmable processor to:

receive separation information representing a separation of one or more of a plurality of peptides of a first peptide mixture over a period of time;

receive mass-to-charge analysis information for one or more of the separated peptides of the first peptide mixture at a particular time in the period of time;

identify one or more of the mass analyzed peptides based on the mass-to-charge analysis information for the peptides;

calculate chromatogram peak areas for the identified peptides;

calculate chromatogram peak areas for one or more proteins corresponding to the identified peptides based on the calculated peak areas for the corresponding peptides;

normalize the chromatogram peak area for the protein based on a chromatogram peak area for an internal standard; and

determine a relative quantity for a protein of the one or more of the proteins by comparing the normalized chromatogram peak area for the protein to a chromatogram peak area for a corresponding protein in a reference sample.

38. Apparatus for quantifying one or more peptides in a first peptide mixture, the apparatus comprising digital circuitry configured to perform the following actions:

receive separation information representing a separation of one or more of a plurality of peptides of a first peptide mixture over a period of time;

receive mass-to-charge analysis information for one or more of the separated peptides of the first peptide mixture at a particular time in the period of time;

calculate an abundance of one or more of the mass analyzed peptides of the first peptide mixture; and

calculate a relative quantity for the one or more mass analyzed peptides of the first peptide mixture by comparing the calculated abundance of the one or more mass analyzed peptides of the first peptide mixture with an abundance of one or more peptides in a reference sample, the reference sample being external to the first peptide mixture.

39. The apparatus of claim 31, wherein the apparatus comprises a programmable processor and the apparatus is configured by instructions stored in a memory for execution by the processor.

40. Apparatus for quantifying one or more peptides in a first peptide mixture, the apparatus comprising digital circuitry configured to perform the following actions:

receive separation information representing a separation of one or more of a plurality of peptides of a first peptide mixture over a period of time;

receive mass-to-charge analysis information for one or more of the separated peptides of the first peptide mixture at a particular time in the period of time;

identify one or more of the mass analyzed peptides based on the mass-to-charge analysis information for the peptides;

calculate chromatogram peak areas for the identified peptides;

calculate chromatogram peak areas for one or more proteins corresponding to the identified peptides based on the calculated peak areas for the corresponding peptides;

normalize the chromatogram peak area for the protein based on a chromatogram peak area for an internal standard; and

determine a relative quantity for a protein of the one or more of the proteins by comparing the normalized chromatogram peak area for the protein to a chromatogram peak area for a corresponding protein in a reference sample.

41. The apparatus of claim 40, wherein the apparatus comprises a programmable processor and the apparatus is configured by instructions stored in a memory for execution by the processor.

42. A method for quantifying one or more compounds in a biological sample, comprising:

receiving a biological sample containing a plurality of compounds;

separating one or more of the plurality of compounds of the biological sample over a period of time;

mass-to-charge analyzing one or more of the separated compounds of the biological sample at a particular time in the period of time;

calculating an abundance of one or more of the mass analyzed compounds of the biological sample; and

calculating a relative quantity for the one or more mass analyzed compounds of the biological sample by comparing the calculated abundance of the one or more mass analyzed compounds of the biological sample with an abundance of one or more compounds in a reference sample, the reference sample being external to the biological sample.

43. Apparatus for quantifying one or more compounds in a biological sample, the apparatus comprising digital circuitry configured to perform the following actions:

receive a biological sample containing a plurality of compounds;

separate one or more of the plurality of compounds of the biological sample over a period of time;

mass-to-charge analyze one or more of the separated compounds of the biological sample at a particular time in the period of time;

calculate an abundance of one or more of the mass analyzed compounds of the biological sample; and

calculate a relative quantity for the one or more mass analyzed compounds of the biological sample by comparing the calculated abundance of the one or more mass analyzed compounds of the biological sample with an abundance of one or more compounds in a reference sample, the reference sample being external to the biological sample.